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Pharmacological Approaches

Principal Investigator: BAUDRY, MICHEL
Grant Number: 1R01NS048521-01A1
Title: Calpain inhibitors in models of Parkinson's disease

Abstract: Parkinson' s disease is a neurodegenerative disease that specifically affects dopaminergic neurons in the substantia nigra. Although several hypotheses have been proposed to account for the specificity of the neurodegenerative features of the disease, the exact cause of the disease remains to be elucidated. Significant advances in our understanding of the possible causes of the disease were provided by the serendipitous discovery that a neurotoxin, 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP), elicits a pattern of neurodegenerative features in humans and experimental animals identical to that seen in patients with Parkinson' s disease. A potential target to prevent neurodegeneration in Parkinson' s disease is the calcium-dependent protease calpain. Calpain levels are elevated in post-mortem substantia nigra of patients with Parkinson' s disease, MPP+ neurotoxicity in granule cell cultures is associated with calpain activation and blocked by calpain inhibitors, and calpain has been implicated in several neurodegenerative diseases. We have recently obtained a series of novel and potent calpain inhibitors and have demonstrated their potency in preventing NMDA-induced calpain activation in cultured hippocampal slices. The current proposal is aimed at testing the hypothesis that calpain activation plays a critical role in animal models of PD and that calpain inhibitors are neuroprotective in these models. We will first determine the potency and efficacy of calpain inhibitors to prevent MPTP toxicity in cultured slices from rat mesencephalon. We will then use structure activity relationship in conjunction with additional assays to identify the best inhibitors to be tested in in vivo models. Finally, we will test the hypothesis that calpain is activated and that calpain inhibitors are neuroprotective against MPTP-mediated neurotoxicity and behavioral impairments in vivo in C57BI/6 mice, and against rotenone-mediated neurotoxicity in rats. Conversion of the pro-apoptotic factor Bid to its active, truncated form tBid will be tested as part of the mechanisms by which calpain activation induces cell death. These studies will test the hypothesis that calpain inhibitors might prevent neurodegeneration not only in Parkinson' s disease but also in a variety of conditions resulting from exposure to environmental toxins. Finally, because calpain has also been implicated in the mechanisms underlying Amyotrophic Lateral Sclerosis (ALS), our proposal could lead to significant advances in the treatment of this neurodegenerative disease as well. -

Principal Investigator: BING, GUOYING
Grant Number: 5R01NS044157-02
Title: Cox-2 deficient mice are resistant to MPTP neurotoxicity

Abstract: Parkinson's disease (PD) is a movement disorder characterized by the progressive loss of dopamine-containing neurons in the substantia nigra pars compacta (SNpc). Loss of SNpc dopaminergic neurons results in the depletion of striatal dopamine levels and produces symptoms such as tremor, muscle rigidity, and bradykinesia. The etiology of PD is unknown, but chronic inflammatory processes, microglial activation, and oxidative stress are thought to play prominent roles in the degeneration of dopaminergic neurons in the SNpc. Microglia are thought to contribute to neurodegeneration by releasing cytotoxic agents such as pro-inflammatory cytokines and reactive oxygen species that increase inflammation and oxidative stress. N-Methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) is a neurotoxin found to mimic many of the features of PD in animal models, including loss of dopaminergic neurons in SNpc and activation of microglia. Recent observations indicate that cyclooxygenase-2 (COX-2) deficiency in mice reduces the susceptibility of SNpc dopaminergic neurons to MPTP toxicity and diminishes MPTP-induced microglial activation. The purpose of this study is to test the hypothesis that COX-2-regulated inflammatory processes exacerbate MPTP neurotoxicity by activating microglia and increasing oxidative stress that contributes to the degeneration of dopaminergic neurons in the SNpc. To test this hypothesis, mice deficient in the COX-2 gene will be treated with MPTP to determine the role of COX-2 in MPTP-induced neurodegeneration. Furthermore, wild-type mice will be administered exogenous COX-2 inhibitors prior to MPTP treatment to evaluate the protective effects of COX-2 inhibitors against MPTP neurotoxicity. Following these experiments, dopaminergic neuron survival, microglial activation, striatal dopamine levels, and functional recovery will be assessed. In addition, protein modification, generation of reactive oxygen species, expression of inflammatory cytokines and apoptosis-related genes, and activation of specific signaling molecules will be evaluated to determine the molecular mechanisms by which COX-2 exacerbates MPTP neurotoxicity. The goals of this study are to elucidate the changes in inflammatory processing affected by COX-2 deficiency, to explore the etiology and molecular mechanisms underlying Parkinsonian symptoms in the experimental MPTP model, and to develop novel therapeutic treatments for PD and other neurodegenerative diseases.-

Principal Investigator: Bohn, Martha C
Grant Number: 5R01NS031957-08
Title: GENE THERAPHY FOR PARKINSON'S DISEASE

Abstract: The long-term goal of this project is to develop novel gene therapies for neurodegenerative diseases. In the previous support period, we focused on adenoviral (Ad) vectors to deliver the gene encoding GDNF (glial cell line-derived neurotrophic factor). Ad-GDNF injected into either the substantia nigra or striatum of a progressive degeneration model of Parkinson's disease protected dopaminergic (DA) neurons against cell death induced by the neurotoxin 6-OHDA. Ad-GDNF injected into the striatum also prevented the acquisition of behaviors and molecular changes that occurred in DA deficient young and aged rats. This proposal focuses on the hypothesis that anti-apoptotic gene delivery will also protect DA neurons in vitro and in vivo and have a synergistic effect with delivery of neurotrophic factor genes. Viral vectors harboring genes that block specific apoptotic death pathways, including XIAP, a dominant-negative caspase-9, bcl-2 and bclxl will be studied for effects on survival and function of DA neurons either alone or in combination with neurotrophic factors, GDNF or neurturin. Genes will be delivered to DA neurons in culture and in rat brain using helper free HSV:AAV hybrid amplicon vectors. These vectors will incorporate bidirectional expression cassettes that drive both the therapeutic gene and the cellular marker gene, green fluorescent protein, to permit specific evaluation of transduced cells. Expression will be controlled using the tetracycline responsive element such that transgene expression is "on" in the presence of tetracycline activator (TA) and in the absence of doxcycline (Dox). Vectors will be made in which TA is driven by a viral promoter of the DA cellular promoter, tyrosine hydroxylase (TH). Effects of the 'therapeutic' genes will be studied using non-neuronal cells, the DA cell line, MN9D, and primary fetal DA neurons treated with the neurotoxins, MPP+ or 6-OHDA or other cellular insults. In vivo effects of therapeutic genes will be studied in: 1) rats that have received grafts of fetal DA neurons, and 2) rats that have received a progressive 6-OHDA lesion of the nigrostriatal projection. Reversibility of effects will be studied by administration of Dox. Effects on DA neurons will be evaluated using quantitative morphometric and molecular techniques and behavioral evaluations. This project also aims to continue its evaluation of new generation viral vectors, including E2b deleted Ad, totally gutted Ad, and HSV:AAV amplicon, for stability and levels of expression in the nigrostriatal system. The studies involve collaborations among investigators at Children's Memorial Hospital and Northwestern Univ. Med. School and are relevant to the development of novel therapies for neurodegenerative diseases and injuries to the CNS. -

Principal Investigator: BURKE, ROBERT E
Grant Number: 2P50NS038370-06
Title: Mechanisms of dopamine neuron degeneration

Abstract: Parkinson's disease (PD) is a prevalent and disabling neurological disease characterized by the progressive loss of motor control due to the degeneration of dopamine (DA) neurons of the substantia nigra. Among neurodegenerative diseases, PD has served as a model for the development of novel therapeutic approaches: administration of neurotransmitter precursors (levodopa), cell implantation, and more recently, deep brain stimulation. As important and effective as these advances have been, they only relieve symptoms; none stop the progression of the disease. In order to develop therapies which halt the progression of the disease, we need to achieve a better understanding of the pathogenesis of DA neuron degeneration. This submission represents a competing continuation application for a Morris K. Udall Parkinson's Disease Research Center of Excellence awarded to Columbia University in 1999. This renewal consists of four projects devoted to a single integrating theme: to understand the molecular and cellular mechanisms of dopamine neuron degeneration. While there are many worthy hypotheses of pathogenesis, the subprojects of this proposal will focus on four major current themes in the pathogenesis of PD, related to the roles of: (1) Abnormal intracellular protein degradation; (2) Inflammatory pathways; (3) Programmed cell death (PCD); and (4) Oxidative injury. In Project 1, Dr Serge Przedborski will evaluate the role of cyclooxygenase 2 (COX2) and cytosolic phospholipase A2 (cPLA2) (Theme 2) in mediating dopamine neuron damage in the MPTP model of PD and in human brain samples. In Project 2, Dr David Sulzer will examine in astrocyte and neuron primary cultures the role of chaperone mediated autophagy in the degradation of proteins implicated in PD (Theme 1) and the effect of these proteins on catecholamine sequestration (Theme 4). In Project 3, Dr Robert Burke will use genetic techniques in animal models to examine the roles of the mixed lineage kinases, Akt and JNK in mediating PCD in dopamine neurons (Theme 3), and he will evaluate the functional role of ER stress in initiating cell death (Theme 1). In Project 4, Dr Lloyd Greene will continue to evaluate the functional role of genes identified in the current funding period by SAGE analysis as upregulated following neurotoxin exposure. He will continue his studies of the role of ER stress-related genes (Theme 1) and genes implicated in PCD (Theme 3) in PC12 cells and primary sympathetic neurons, and in living animal models (the latter in collaboration with Drs Burke and Przedborski). He will also examine these transcripts and their protein products in PD brain. -

Principal Investigator: CANAVIER, CARMEN C
Grant Number: 5R01NS037963-06
Title: Firing Pattern in Midbrain Dopamine Neurons

Abstract: This work seeks to understand the how the synaptic afferent inputs to midbrain dopamine neurons interact with their intrinsic properties to produce the range of firing patterns exhibited in vivo, and how these firing patterns exert their effects on the target neurons in the striatum. We will first produce a computer model of the dopamine neurons in vitro that replicates the effects of pharmacological manipulations on the regular spontaneous firing that characterizes dopamine neurons in the absence of afferent input, and provides insight into the mechanisms that convert this regular firing into burst firing or irregular firing. Then we will extend the model to the situation in vivo. The model will be used not only to elucidate the key currents, parameters, and mechanisms responsible for the generation and modulation of their electrical activity, but also to suggest therapeutic approaches for Parkinson's disease and other pathological conditions in which dopamine release plays a role. Currently such therapeutic strategies, including maximizing release from surviving or transplanted dopamine neurons, are limited by the inability to replace dopamine in the correct spatial and temporal pattern. Several lines of evidence indicate that not only the firing rate but also the firing pattern of these neurons is significant. Computational models supplemented by the techniques of nonlinear forecasting and nullcline analysis, will used to test our hypotheses about how various pharmacological agents exert their effects on the firing pattern of dopamine neurons, and how these changes in firing pattern might impact their targets in the striatum. We will identify model mechanisms and parameters responsible for characteristics of apamin and NMDA-induced burst firing such as variations in spike amplitude and interspike interval (ISI) as well as depolarization block, identify mechanisms responsible for irregular firing both in the model and in real neurons in vivo and in vitro, formulate a model of burst firing induced by synaptic excitation in vivo, and test our hypotheses regarding the functionality of irregular firing and the role of Dl receptor activation in focusing striatal activity. -

Principal Investigator: CARTER, JULIE H
Grant Number: 3U10NS044483-02S1
Title: Parkinson's Disease Neuroprotection Clinical Trial

Abstract: Unavailable

Principal Investigator: Chase, Thomas
Grant Number: 5Z01NS002265-28
Title: Pathogenesis And Treatment Of Neurodegenerative Disease

Abstract: Unavailable

Principal Investigator: CHEN, JIANG F
Grant Number: 5R01NS041083-05
Title: NOVEL BENEFIT OF A2A RECEPTOR INACTIVATION IN PD MODELS

Abstract: (Adapted from the Applicant's Abstract) Parkinson's disease patients experience profound depletion of striatal dopamine (DA) due to degeneration of the nigrostriatal DA pathway. The predominant treatment for the past 30 years has been the DA precursor, L-dopa. While this strategy improves motor deficits, it has no effect on the underlying degenerative process, and indeed can have the additional unwanted side-effect of inducing dyskinesia. A possible alternative therapy, with neuroprotective ability appears to be use of antagonists of a specific class of adenosine receptors, A2A. These agents appear to have both motor-activating properties and preliminary data suggest they may also attenuate MPTP-induced DA neurotoxicity and prevent the locomotor stimulation that occurs with chronic DA receptor stimulation. The proposed studies will systematically investigate the novel motor and neuroprotective effects of A2A receptor antagonists. Methods center around pharmacological studies and use of genetic knockout (KO) approaches. There are three specific aims: 1) to test the hypothesis that A2A inactivation enhances motor function through D2R-dependent and independent mechanisms using A2AR-KO, D2R-KO and double KO mice; 2) to test the hypothesis that A2AR inactivation prevents the development of chronic L-dopa-induced rotational motor sensitization in unilateral 6-OHDA-lesioned mice; and 3) to characterize the role of V in MPTP-induced neurotoxicity by establishing the potency, "therapeutic window" and by "analyzing synergy between A2AR activation and inactivation;" in addition, the effect of A2AR agents on MPTP metabolism in vivo and in cell culture will also be examined to investigate the neurochemical mechanisms of protection by A2AR inactivation. -

Principal Investigator: Dani, John A
Grant Number: 1R01NS048505-01
Title: Simulation-guided Nicotinic Synapse & AD Drug Mechanisms

Abstract: Cholinergic systems provide diffuse innervation to nearly every area of the brain, and drive or modulate a wide variety of behaviors. Nicotinic acetylcholine receptors (nAChRs) have been implicated in many diseases, such as epilepsy, addiction, schizophrenia, Parkinson's disease, vascular dementia, dementia with Lewy bodies, and Alzheimer's disease. In this application, we propose experiments that are interactively coupled to computer models of synaptic function. The computer models are intended to be generalized tools that enhance the experimentalist's insights into basic synaptic mechanisms, pharmacology, and disease. We are initiating this approach by focusing on cholinomimetic drugs at nicotinic synapses. Such drugs are now the only approved treatments for mild to moderate Alzheimer's disease (AD). The working hypothesis is that the cholinergic drugs have varied mechanistic effects at nicotinic cholinergic synapses, and by affecting nAChRs, these drugs also influence the release of other neurotransmitters. Our aim is to implement this approach with three levels of interactive modeling and experimentation. At each level we have used data from the literature to develop preliminary computer models. The models produce simulations that guide the design and interpretation of the experiments. At the first level of interaction between simulations and experimentation, we apply patch-clamp electrophysiology in tissue culture and slice to determine activation and desensitization parameters for the nAChRs. These basic data supplement those from the literature, enabling us to develop reliable models of nAChR kinetics that will be used throughout this research. At the second level of interaction, we use electrophysiology to examine the pharmacology of cholinomimetic drugs at nicotinic synapses. The experiments are guided and the interpretation assisted by a model of a CNS nicotinic synapse. At the third level, we use cyclic voltammetry in striatal brain slices to examine cholinergic/dopaminergic interactions guided by a model of interacting CNS synapses. The work proposed here will serve as the basis for future extensions to network interactions among neurotransmitter systems and future applications to other neurological diseases. -

Principal Investigator: DAWSON, TED M
Grant Number: 2P50NS038377-06A1
Title: Parkinson's Disease Research Center of Excellence

Abstract: The overall goals of this proposal are to understand the role of alpha-synuclein, parkin, DJ-1 and synphilin-1 in the pathogenesis and pathology of Parkinson's disease (PD) and to define the molecular mechanisms of neuronal injury in animal models of PD. The program represents a multi-disciplinary, mechanistic approach involving interactive, productive investigators with complementary areas of expertise who have long been committed to the studies of neurodegenerative diseases. Their aim will be to integrate the activities of various disciplines such that the interrelationships will result in a greater scientific contributions and achievements if each project were pursued individually. The program has one major theme: To understand the role of familial associated genes alpha-synuclein, parkin and DJ-1 in the pathogenesis of Parkinson's disease and related disorders. The role of alpha-synuclein, parkin, DJ-1 and synphilin- 1 in PD pathogenesis will be investigated using molecular, transgenic, neuropathologic, cell biologic and neurobehavioral approaches to examine the mechanism of neuronal dysfunction and injury clue to alterations in these gene products. The mechanism of neuronal loss in Parkin knockout mice and alpha-synuclein A53T transgenic mice will be characterized. We will determine whether parkin interacts with alpha-synuclein and further explore the relation between and parkin, alpha-synuclein and synphilin-1. We will explore alpha-synuclein processing and modifications and the relationship of synphilin-1 to alpha-synuclein. Furthermore, we will investigate the potential function of DJ-1 and it role in PD Pathogenesis. We believe that our multi-disciplinary approach has the capacity to produce unique information concerning the mechanisms of neurodegeneration in genetic animal models of Parkinson's disease and the related synucleinopathies and to lead to better understanding of the function and the role of alpha-synuclein, parkin, DJ-1 and synphilin-1 in normal and pathophysiologic processes related to PD. The program consists of four projects: 1) Mouse Models of Parkin Biology and Pathobiology 2) PD Cell Models: Alpha-synuclein and Interacting Proteins; 3) Mechanisms of Neurodegeneration in Human Alpha-synuclein Transgenic Mice; 4) The Role of DJ-1 in Parkinson's Disease and four cores A) Administration and Training; B) Transgenic and Neurobehavior; C) Neuropathology and D) Clinical.-

Principal Investigator: DEUTCH, ARIEL Y
Grant Number: 5P01NS044282-03
Title: Dendritic Plasticity in Parkinson's Disease

Abstract: A decrease in striatal dopamine (DA) concentration underlies Parkinson's disease (PD). DA terminals synapse onto striatal medium spiny neurons (MSNs), forming a triad with corticostriatal glutamatergic synapses; the excitatory cortical input is typically onto the head of the dendritic spine and the DA synapse onto the spine neck. DA is thereby critically positioned to gate excitatory glutamatergic inputs to MSNs. A variety of compensatory mechanisms are set into play by decreased striatal DA levels and attempt to maintain normal function in the face of progressive DA loss. Certain changes may afford some benefit but may ultimately be counterproductive. 6-hydroxydopamine (6-OHDA) lesions of the striatal DA innervation result in decreased dendritic spine density and decreased dendritic length in MSNs; a similar picture has been reported in PD. Glutamate has been shown to regulate spine formation and maintenance through NMDA and AMPA receptors, respectively. We hypothesize that striatal DA depletion results in decreased dendritics spine density by increasing glutamatergic transmission, which causes an increase in intracellular calcium levels. The increase in [Ca2+]i results in spine shortening and loss, thus delimiting excitatory drive onto the MSN. However, these dendritic changes may also limit the effectiveness of DA replacement treatment through loss of dendritic spines, on which DA receptors reside. This programmatic effort will test this hypothesis through four projects. The first project will determine if loss of DA tone at the D2 receptor is responsible for the dendritic changes in MSNs, and test the hypothesis that calcium influx through L-type calcium channels is an effector. The second project examines the dopaminergic regulation of CaMKII in the MSN; this dendritically-transcribed Ca2+-dependant enzyme regulates phosphorylation of the GluRl subunit of the AMPA receptor and 2B subunit of the NMDA receptor, and is thus a key to effectiveness of excitatoty glutamatergic transmission. The third project tests the hypothesis that calcineurin (PP2b), a Ca2+- activated phosphatase recruited by dopamine signaling through the D2 receptor, regulates glutamatergic drive onto MSNs and will determine if the decrease in spine density in MSNs is altered by genetic up- or down-regulation of PP2b. The final project will determine changes in dendritic morphology at both the light and electron microscopic level in postmortem material from PD patients and correlate dendritic changes with clinical status; this work will also determine the forms of synaptic reorganization that accompany spine loss. These projects should shed light on the pathophysiology of PD and may lead to development of new strategies aimed at slowing or

Principal Investigator: ESTEVEZ, ALVARO G.
Grant Number: 5R01NS036761-07
Title: PEROXYNITRITE AND SOD IN MOTOR NEURON APOPTOSIS

Abstract: Our long-term goal is to understand how mutations to SOD can increase oxidative stress and cause the death of motor neurons in amyotrophic lateral sclerosis (ALS). We have shown that endogenous formation of the peroxynitrite by the diffusion-limited reaction between superoxide and nitric oxide induces apoptosis in cultured embryonic rat motor neurons deprived of trophic support. Both inhibitors of nitric oxide synthesis as well as Cu, Zn superoxide dismutase (SOD) delivered intracellularly with liposomes protect motor neurons from apoptosis. These data indicate that the interaction between nitric oxide and superoxide has a role in motor neuron apoptosis. Mutations to SOD are implicated in the selective degeneration of motor neurons in ALS and expression of ALS-SOD mutants in transgenic mice produces motor neuron disease. A common phenotype among the ALS-SOD mutations so far investigated is to decrease the affinity for zinc. We have shown that zinc-deficient SOD is both less efficient at scavenging superoxide and a better catalyst of tyrosine nitration. Furthermore, the copper in zinc-deficient SOD can act as a non-specific one-electron oxidase, robbing electrons from antioxidants like ascorbate and glutathione that can be transferred to oxygen to produce superoxide. In the presence of NO, zinc-deficient SOD can catalyze the formation of peroxynitrite. In the previous cycle of funding, we have shown that zinc-deficient SOD induces apoptosis in motor neurons by a nitric oxide-dependent mechanism. For the renewal, our first aim is to further investigate the mechanisms by which zinc-deficient SODs can kill cultured motor neurons and to determine what can protect motor neurons from this toxicity. Our second aim is to characterize the source or sources of superoxide induced in motor neurons by trophic factor is to characterize the source or sources of superoxide induced in motor neurons by trophic factor withdrawal. Our third aim is to test the role of tyrosine nitration by peroxynitrite in the death of motor neurons induced by either trophic factor deprivation or by zinc-deficient SOD. Completion of the specific aims will provide a mechanistic basis for explaining how motor neurons are particularly vulnerable to SOD mutations and establish a link between sporadic and familial SODs. -

Principal Investigator: GERHARDT, GREG A
Grant Number: 3P50NS039787-05S1
Title: RESTORATION OF DOPAMINE FUNCTION IN PARKINSON'S DISEASE

Abstract: Unavailable

Principal Investigator: Goldstein, David
Grant Number: 5Z01NS002979-06
Title: Clinical Neurocardiology: Catecholamine Systems In Stress And Disease

Abstract: Unavailable

Principal Investigator: HAMILL, ROBERT W
Grant Number: 3U10NS044501-02S1
Title: Parkinson Disease Neuroprotection Clinical Trial

Abstract: Unavailable

Principal Investigator: HERSHEY, TAMARA G
Grant Number: 5K23NS041248-04
Title: Dopaminergic Modulation of Working Memory in PD

Abstract: The applicant is a clinical neuropsychologist with graduate training in neuropsychology and postdoctoral training in neuropharmacology and positron emission tomography (PET). The goal of this career development award is to integrate and advance these two areas of interest to answer questions about the neuropharmacological and neurophysiological basis of cognitive dysfunction in movement disorders such as Parkinson's disease (PD). This award will provide the applicant with training in the technical and theoretical issues related to using cognitive and pharmacological activation techniques in functional magnetic resonance imaging (fMRI). Long-term objectives are to address questions about the neural basis of cognitive dysfunction in movement disorders related to dopaminergic and/or basal ganglia dysfunction, such as PD, Tourette's syndrome and Huntington's disease. In addition, questions about the effects of dopaminergic treatments for these and other disorders (e.g. dystonia) on cognitive and neurophysiological functioning are also of interest. Cognitive dysfunction in these diseases, either due to the disease process itself or its treatments, can be limiting and disabling. Understanding the neurophysiologic basis for these symptoms may aid in assessing the effectiveness of current treatments or in developing better treatments. During the award period, the applicant will develop expertise in the use of fMRI, cognitive and neuropharmacological techniques to study these disorders, and will continue to hone her clinical skills in the neuropsychological assessment of movement disorders. The applicant will apply these new techniques to investigate the role of dopamine in working memory. The specific aims of the proposed studies are to test the hypothesis that 1) PD affects prefrontal cortex involvement in working memory and 2) dopaminergic modulation of working memory primarily occurs due to changes in lateral prefrontal cortical activity. To test these hypotheses, the applicant will first perform a behavioral study examining the effects of a steady-state infusion of levodopa, a dopamine precursor, on verbal and spatial working memory in PD patients and controls. The results of this study will then guide the choices of working memory tasks for an fMRl study. Subjects will be asked to perform working memory tasks before and during a steady-state infusion of levodopa. Modulation of the lateral prefrontal cortex is predicted during levodopa infusion. The degree of modulation is predicted to depend on baseline dopaminergic status (PD vs control) and the degree of memory load (low vs high). -

Principal Investigator: JAEGER, DIETER
Grant Number: 5R01NS039852-05
Title: CONTROL OF SPIKING IN BASAL GANGLIA OUTPUT NEURONS

Abstract: The objective of the proposed research is to determine how the activity of neurons in the substantia nigra pars reticulata (SNr), one of the major output nuclei of the basal ganglia, is controlled by synaptic input. Simple network models of basal ganglia function and disorders assume that the activity of output neurons is determined by summing the amount of inhibitory and excitatory inputs received. It is clear, however, that single neurons have active intrinsic mechanisms by which synaptic inputs may be integrated in a highly complex non-linear fashion. These complex properties of synaptic integration will be examined in SNr neurons by combining in vitro whole cell recording, extracellular recording and computational modeling. First the passive and then the active properties of these neurons will be catalogued using whole-cell recordings in rat brain slices. These experiments will use current and voltage-clamping in conjunction with pharmacological blockade of various voltage- and ligand gated channels to isolate and characterize purely passive membrane properties and specific voltage-dependent conductances. Recorded neurons will be intracellularly stained and reconstructed histologically with Neurolucida, and the quantitative morphometric data obtained will be used along with the electrophysiological data to construct a compartmental model of SNr neurons. The model will be adjusted and fine tuned by comparing the behavior of the model to that of SNr neurons in whole cell recordings in vitro and in extracellular single unit recordings in vivo, while constraining the parameters to those obtained in the recording experiments. To study the mechanisms by which synaptic input controls activity, the parameters of synaptic inputs including the time courses and amplitudes of excitatory and inhibitory inputs will be measured and used in the model. Finally, realistic sequences of synaptic input, inferred from in vivo and in vitro recordings of SNr neurons will be input to the model to determine the input-output function of SNr neurons. -

Principal Investigator: KEEFE, KRISTEN A
Grant Number: 5R01NS041673-03
Title: Regulation of Striatal Neurons by NMDA Receptor Subtypes

Abstract: Parkinson's Disease (PD) is a devastating movement disorder consequent to massive death of neurons in the substantia nigra. An important functional consequence of this cell death is depletion of the neuromodulator dopamine (DA) within the striatum. In addition to the DA input, the striatum also receives major glutamate input from the cerebral cortex and thalamus. The ameliorative effects of DA agonists in animal models of PD are potentiated by antagonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. In addition, NMDA antagonists block the appearance of dyskinesias in Parkinsonian animals treated with DA agonists and also block DA agonist and antagonist-induced immediate early gene expression in the intact striatum. Recent data from our laboratory indicate that distinct NMDA receptors selectively interact with Dl and D2 DA receptors to regulate immediate early gene expression in striatonigral and striatopallidal efferent neurons in both DA-depleted and intact animals. Furthermore, evidence suggests that corticostriatal and thalamostriatal afferents selectively affect the function of D2 receptor-containing striatopallidal and Dl receptor-containing striatonigral neurons, respectively. Finally, data from studies on other brain regions indicate that different NMDA receptor subtypes can be targeted to synapses associated with distinct afferent pathways within the same neuron. Thus, the goal of this proposal is to test the hypothesis that Dl and D2 dopamine receptors selectively interact with distinct NMDA receptors by virtue of the afferent-selective expression of distinct post-synaptic NMDA receptors in striatal efferent neurons. This hypothesis will be tested by completing the following specific aims: A) Establish how generalized the selective association of specific NMDA and DA receptors is across striatal efferent neuron responses. B) Determine the pharmacology of NMDA receptors mediating corticostriatal and thalamostriatal activation of immediate early gene expression in striatal efferent neurons in vivo and examine the modulation of this activation by DA receptor manipulations. C) determine the kinetics and pharmacology of NMDA receptor-mediated EPSCs evoked in striatal efferent neurons by activation of cortical and thalamic afferents and examine the modulation of those EPSCs by DA receptor manipulations. It is anticipated that the results of these experiments will provide new insight into the functional relationship between DA and NMDA receptors in the regulation of striatal efferent neurons and will lead to important new advances in therapeutic interventions for the treatment of PD and other disorders of the basal ganglia. -

Principal Investigator: KIEBURTZ, KARL D.
Grant Number: 5U01NS043128-04
Title: Neuroprotection Studies in PD: A Coordinating Center

Abstract: Unavailable

Principal Investigator: KOPIN, ALAN S
Grant Number: 5R21NS043692-02
Title: Evaluation dopamine receptors in Parkinson's

Abstract: Parkinson's disease (PD) results from the degeneration of nigrostriatal dopaminergic neurons. This process ultimately leads to a progressive decrease in dopamine mediated striatal signaling which manifests as a clinical syndrome characterized by bradykinesia, rigidity, tremor, and gait abnormalities. Traditional therapy for PD has aimed at restoring dopamine levels in the striatum through administration of the dopamine precursor, L-dopa. With advanced disease, L-dopa leads to dyskinesias and periods of marked fluctuation in motor activity ('on-off effect'). Alleviation of these side effects has been a major challenge and has prompted a search for alternative strategies which can provide a more stable level of dopaminergic signaling. A previously unexplored option to restore striatal dopaminergic activity and at the same time to potentially avoid the consequences of long term L-dopa administration, is through the introduction of constitutively active dopamine receptors. The laboratory of the PI has extensive experience in generating receptors with ligand independent (or constitutive) activity through the introduction of activating point mutations. These receptors have the potential to maintain dopaminergic signaling even in the absence of dopamine and/or dopaminergic agonist drugs. The premise of this application is that constitutively active dopamine receptors can be identified using in vitro assays and expressed in the striatum of rats to enhance dopaminergic signaling over an extended time interval. The objective of Specific Aim 1 is to generate and pharmacologically characterize in vitro a series of constitutively active dopamine 1 and dopamine 2 receptors. Using recombinant adeno-associated virus, the functional consequences of striatal overexpression of constitutively active dopamine receptors will be explored in rats (Specific Aim 2). Circling behavior after unilateral viral administration will be used as an index of construct activity. The methodologies utilized will include molecular (generation of constitutively active mutant receptors, expression of recombinant proteins), pharmacologic (radioligand binding, second messenger signaling assays), and behavioral approaches (assessment of circling behavior). These experiments will provide additional insight into the role of dopaminergic receptors in the striatum as well as potentially take the first steps toward the development of a new therapeutic option for Parkinson's disease. -

Principal Investigator: LANGSTON, J W
Grant Number: 5R01NS034886-07
Title: MECHANISMS OF DOPA-DYSKINESIAS IN PARKINSONIAN MODELS

Abstract: This is the first competitive continuation of an ongoing NIH application to investigate dopa-dyskinesias in parkinsonian MPTP-lesioned monkeys. The long-term goal of this work is to elucidate the mechanisms underlying this devastating complication of chronic L-dopa therapy, which is a major barrier for the successful treatment of Parkinson's disease. During the course of our ongoing grant, we unexpectedly observed that normal animals also developed dopa-dyskinesias, in contrast to previous work which suggested that a nigrostriatal deficit is essential for this complication of L-dopa therapy. This new non-lesioned model of dopa-dyskinesias may provide insight concerning the etiology of these movement abnormalities because it allows us to investigate this phenomenon in a setting that is not confounded by an already damaged nigrostriatal system. By examining the biochemical changes caused by L-dopa in unlesioned as compared to MPTP-lesioned animals, we should be able to identify common molecular mechanisms that underlie the development of typical dyskinesias. In this competitive continuation, the behavioral, cellular and molecular mechanisms associated with L-dopa-dyskinesias will be studied. This will be approached by (1) testing the hypothesis that a compromised nigrostriatal dopamine reuptake system predisposes to dopa-dyskinesias. This will be studied by initiating a drug-induced impairment of dopamine reuptake in normal animals to determine if there is an enhanced susceptibility to dopa-dyskinesias. (2) We will also investigate the relative roles of dopamine receptor subtypes (D1, D2 and D3) in the genesis of dopa-dyskinesias by administration of receptor subtype specific agonists and antagonists. (3) Our third specific aim will involve experiments to determine the integrity of the nigrostriatal system in the different groups of monkeys with dopa-dyskinesias. (4) Lastly, we will study the molecular events in the basal ganglia which mediate the development of dopa-dyskinesias using different models of dyskinesias described above. This will include alterations in dopamine receptor-linked coupling mechanism (such as dopamine-stimulated 35SGTPgammaS binding, DARPP-32 phosphorylation and adenylate cyclase activity), changes in NMDA receptor number and phosphorylation, and alterations in PPE mRNA levels. The results of this work will advance our understanding of the molecular mechanism responsible for the debilitating dyskinetic movements which occur as a consequence of long-term L-dopa treatment in Parkinson's disease. -

Principal Investigator: LEEHEY, MAUREEN A
Grant Number: 3U10NS044479-02S1
Title: U Colorado Parkinson's Disease Clinical Research

Abstract: Unavailable

Principal Investigator: LI, SENLIN
Grant Number: 1R01NS046004-01A1
Title: Macrophage Gene Therapy of Neurodegenerative Diseases

Abstract: Neurodegenerative diseases affect a large population of patients. Existing therapies are not satisfactory. Gene therapy holds promise, but focal delivery of DNA and the level of gene expression are challenging. Macrophages are recruited from bone marrow to most tissues of the body including the CNS, thus making them an attractive option for gene delivery. Galactosialidosis (GS) has been corrected by bone marrow-derived macrophages expressing human protective protein/cathepsin A (PPCA) transgene in a mouse model (PPCA-/-). However, correction in the CNS was incomplete due in part to weakness of the CSF-1R promoter used in the study. We have developed a series of super macrophage promoters (SMP) that are up to l00-fold stronger in vitro than the CSF-1R promoter. In models of the highly prevalent Parkinson's disease (PD), local delivery of glial cell line-derived neurotrophic factor (GDNF) has been found beneficial. We hypothesize that highly effective CNS delivery of GDNF can be achieved with the use of our super macrophage promoters and this will greatly ameliorate the pathologic changes and neurological defects in animal models of PD. To explore this hypothesis, our specific aims are: 1) To characterize these super macrophage promoters by transplantation of bone marrow stem cells transduced ex vivo with lentiviral vectors and in transgenic mice using EGFP (enhanced green fluorescent protein) as a reporter. Promoters with the greatest strength and tissue-specificity for macrophages will be used in the subsequent aims. 2) To ameliorate neurodegeneration in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease by syngeneic transplantation of HSC transduced ex vivo with lentivectors expressing GDNF gene in macrophages/macroglia driven by the SMP. Bone marrow stem cells will be transduced ex vivo with GDNF expressing lentivirus and transplanted into lethally irradiated recipient mice. Four weeks after bone marrow transplantation, the recipient mice will be injected subcutaneously with MPTP. At selected time points post MPTP administration, PET scan and behavioral testing will be performed, and brain tissue will be examined for dopamine uptake and expression of tyrosine hydroxylase (TH). In the substantia nigra pars compacta (SN), dopaminergic neurons will be counted and cell apoptosis will be assessed by TUNEL staining and immunohistochemistry for active easpase-3. 3) To ameliorate neurodegeneration in the same way as in Aim 2, but GDNF expression will be controlled by a tetracycline-regulatable gene expression system. To evaluate the effects of macrophage/ super promoter-mediated delivery and expression of GDNF on degenerating

Principal Investigator: MAILMAN, RICHARD B
Grant Number: 5R01NS039036-05
Title: MOLECULAR REGULATION OF D1 DOPAMINE RECEPTOR FUNCTION

Abstract: This is a revised application based on data showing that the first full D1 dopamine agonist dihydrexidine that we developed caused profound acute antiparkinsonian effects in primates. Recent data indicate that some, but not all, full D1 agonists produce marked tolerance when administered repeatedly. We also have shown that D1 dopamine receptor agonists of similar efficacy can differ dramatically in desensitization liability. Using a series of rigid Dl agonists, we shall determine if selective conformations are promoted by different full Dl agonists, and whether these conformations represent different, if overlapping, receptor states compared to those that promote G protein coupling. First, we shall study differences in how novel D1 agonists functionally desensitize hemagglutinin (HA)-tagged human D1 receptors (HA-D1R) in C-6 glioma cells as affected by the extent and/or pattern of GRK activity. The rate and extent of D1 receptor phosphorylation by different agonists, and the effects of dominant negative GRK mutants, will be determined. Molecular and pharmacological tools will be used to assess the relative roles of endogenous GRKs and PKA in such desensitization. We also shall determine the cellular response of GRK to agonist exposure by assessing its translocation and interaction with G-beta-gamma complements. Second, we shall map the phosphorylated residues of the hD1R receptor. Patterns of agonist-induced receptor phosphorylation induced by GRKs and second-messenger-kinases (e.g., PKA) will be assessed by mutating subsets of serines and threonines in HA-hDl-C-6 cells. We also shall overexpress HA-D1R with and without various GRKs in HEK293 cells. The phosphorylated HA-hD1 receptor will be isolated by immunoprecipitation, cleaved with protease, and sequenced by MALDVToF, Ion Trap, and/or nano-ESI-mass spectrometry. Finally, we shall examine the relationship between GRK activity and arrestin binding in the initiation of G protein uncoupling and functional desensitization in the HA-hD1 C-6 system. The complement of Q-arrestins will be determined, and alterations in high affinity binding of GRKs and arrestins to D1 receptors measured following exposure to select D1 agonists. The loss of receptor-G-protein coupling from binding of labeled GTP-analogs will be assessed to correlate receptor uncoupling with levels of)-arrestin binding. The necessity of 5-arrestin(s) in functional desensitization of the HA-hD1 receptor in C-6 cells will be studied using dominant-negative mutants, and the relation between GRK and p-arrestins assessed using tools developed in Aim 1 studies. These studies of beta-arrestin binding and recruitment will provide another functional endpoint in the

Principal Investigator: MENTIS, MARC J
Grant Number: 5K23NS002204-05
Title: MECHANISMS UNDERLYING THERAPY IN PARKINSON'S DISEASE

Abstract: The award is intended to develop the candidate's research skills in, psychophysics, pharmacology, and advanced functional imaging (systems analysis, and fMRI) to equip him for an independent career evaluating mechanisms underlying successful therapy of cognitive dysfunction in neurodegenerative diseases. Once identified, successful medical and/or surgical mechanisms can be manipulated to refine existing, and develop novel therapies. Research Plan: Parkinson's Disease (PD), expected to afflict 1,000,000 Americans by the year 2000, frequently exhibits cognitive deficits (dysexecutive syndrome) in non- demented PD patients, in addition to the 40% with dementia. While the deficits have been linked to frontal cortical dysfunction and/or a disorder of subcortico-frontal connectivity, the functional basis of these deficits in PD remains poorly understood. Dopamine replacement therapy, successful for the motoric signs of PD, falls to improve the dysexecutive syndrome. Pathological studies show 20% loss of cholinergic cells in subcortical nuclei of non-demented PD patients, abnormal cortical choline acetyltransferase, reduced cortical and subcortical nicotinic receptors, and a correlation between cortical nicotinic loss and cognitive dysfunction. Preliminary clinical reports suggest cognitive improvement with non-specific cholinergic therapy. In Specific Aim 1, the candidate proposes a PET study of PD patients performing kinematically-controlled motor learning and execution tasks at baseline and with cholinergic pharmacotherapy. The pharmacological technique will allow him to identify the contribution of receptor families (muscarinic and/or nicotinic) to cholinergic modulation of specific brain networks known to be associated with learning performance. Based on these results, the candidate will test the hypothesis that nicotinic therapy will improve defined aspects of cognitive dysfunction in PD. The candidate wishes to bridge the gap between clinical of cognitive abnormality, and pathological observation of cholinergic loss, firstly, by quantifying the modulation of cholinergic receptor families on brain networks subserving cognition, then by testing if predicted improvements occur with therapy of a particular receptor family. In Specific Aim 2, the candidate will expand upon a preliminary observation that deep brain stimulation (DBS) may improve learning performance in PD. He will perform a PET study on PD patients on and off subthalamic nucleus (STN) DBS to determine the effect of therapeutic stimulation on the same tasks as in Specific Aim l. This will test the hypothesis that STN DBS may enhance cognitive performance in PD by modulating the expression of subcortico-frontal projection

Principal Investigator: MENZA, MATTHEW A
Grant Number: 5R01NS043144-02
Title: Treatment of Depression in Parkinson's Disease

Abstract: Depression is the most common neuro-psychiatric disorder found in patients with Parkinson's Disease (PD). It causes immense personal suffering, and is associated with increased disability and caregiver burden. Despite the adverse consequences of depression in patients with PD, there are virtually no empirical data to guide clinical treatment. In the absence of data, the SSRIs are apparently used as the first-line treatment, despite concerns about efficacy, safety, and tolerability in this population. This proposal is for a pilot study to establish the feasibility of, and generate sufficient data to plan, a larger clinical trial that will be able to inform clinical treatment of these patients. This pilot trial will (AIM 1) examine the feasibility of a larger trial, and establish (AIM 2) the effect size for short-term efficacy of anti-depressants, compared to placebo, in this population. It will also (AIM 3) evaluate the effect of long-term depression treatment on quality-of-life. This will be done in the context of a placebo-controlled, double-blind, parallel group, flexible dose trial of an SSRI (Paroxetine), a tri-cyclic (Nortriptyline) and placebo in acute (8 weeks) and long-term treatment (6 months). A total of 75 patients with PD (without significant motor fluctuations or Dementia) and depression (major depression or Dysthymia) will be randomized to each of the three arms in a balanced design. The feasibility issues that will be explored include recruitment, retention, drug tolerability, and the ability to maintain the blind. The outcomes that will be explored for the acute phase include changes in the Hamilton Depression Rating Scale (HAM-D) score, and the percent of patients who are responders (>50% improvement in the HAM-D, or < 10 on the HAM-D). The outcome variables explored for the long-term phase include the Parkinson's Disease Questionnaire and the Medical Outcome Study Short Form. Secondary analyses will involve the exploration of anxiety, motor disability, sleep, cognition, and individual or clusters of symptoms that are responsive to treatment in order to facilitate planning a subsequent, full-scale clinical trial. -

Principal Investigator: Meredith, Gloria
Grant Number: 5R01NS041799-05
Title: Synaptic Proteins, Trophic Factors and Neurodegeneration

Abstract: One of the most fundamental questions related to the progressive nature of neurodegeneration in human disease is how neurons die. Protecting nerve cells against morphological decline and death requires blocking intrinsic factors that inhibit neural repair. In the present proposal, we offer an innovative approach to study those factors that are active in Parkinson's disease (PD) in a new mouse model that shows synaptic loss and irreversible nigrostriatal degeneration. We propose to track changes of a key synaptic protein, a-synuclein, both in its native environment at presynaptic terminals and under neurotoxic conditions, when it becomes insoluble and accumulates. We will further correlate those changes with altered neurotrophic support. We have established an animal protocol by treating C57/bl mice with a combined regimen of 10 doses of probenecid at 250mg/kg and MPTP at 25mg/kg for 5 weeks. These mice show a slow, progressive loss of nigrostriatal dopaminergic function for at least 6 months, that mimics PD, with no signs of recovery. Three weeks after drug treatment, there is a significant reduction in the number of substantia nigra (SN) cells and dramatic changes in the subsynaptic distribution and density of a-synuclein-immunoreactive terminals. These changes could signal the beginning of a chain of events that leads to cell death. In this proposal, we will focus on the progressive deterioration of dopaminergic neurons in the SN and their inputs, and present three specific aims to be addressed through a series of hypotheses. Specifically, we plan to 1) ascertain the origin and neurochemical phenotype of synapses in the SN that contain a-synuclein and to establish whether MPTP + probenecid treatment leads to their degeneration; 2) determine, in the MPTP+P model, the temporal relationships between cell death and a-synuclein-positive synapses, decline in dopamine function and behavior; and 3) ascertain whether changes in a-synuclein expression and production are precipitated by altered neurotrophic support. The overall objective of our research is to understand the relationship between the synaptic protein, a-synuclein, neurotrophic support, especially brain-derived neurotrophic factor (BDNF) and their respective roles in the PD form of neurodegeneration. The findings of this research should shed light on target areas where neuroprotection strategies can be implemented. -

Principal Investigator: Munzar, Patrik
Grant Number: 2R44NS045505-02
Title: Inhaled dopamine agonists for late stage Parkinsonism

Abstract: Many patients in the later stages of Parkinson's disease experience periods of acute immobility ("off' periods) that substantially decrease their quality of life. The most effective pharmacological treatments for these acute "off' periods are dopamine agonist drugs, which can rapidly abort "off' periods if delivered quickly into the blood stream via injection. The utility of this form of treatment is, however, limited due to its invasiveness and the inability of many late-stage Parkinson's disease patients to self-administer injections. The aim of this project is to develop an inhalation device that delivers dopamine agonists rapidly into the blood stream in a convenient, non-invasive fashion. We have developed a novel drug delivery technology that involves heating drugs such that they vaporize, but do not degrade, and subsequently cool and condense into small particle aerosols suitable for systemic delivery by inhalation. In Phase I of this proposal, we have constructed a handheld device capable of generating pure aerosols of several dopamine agonists, demonstrated the biological activity of these aerosols in vitro, and confirmed that the aerosol's particle size is appropriate for systemic delivery via deep lung inhalation. In Phase II of this grant we will prove that inhaled dopamine agonists rapidly reverse Parkinson's disease symptoms in an animal model and will conduct all pre-clinical work required to initiate clinical development of inhaled dopamine agonist for treatment of late stage Parkinson's disease. Successful completion of these aims will allow us to move into Phase I clinical testing, which will be funded by a combination of outside investors, FDA funds for the development of orphan drugs, and/or a partnership with a major pharmaceutical company. Eventual FDA approval of inhaled dopamine agonist product for treatment of motor fluctuations in late stage Parkinson's will substantially improve the treatment of this serious and common neurodegenerative disease. -

Principal Investigator: Oldfield, Edward
Grant Number: 5Z01NS002813-15
Title: Drug Delivery Techniques

Abstract: Unavailable

Principal Investigator: O'MALLEY, KAREN L
Grant Number: 2R01NS039084-05A1
Title: Mechanisms of Neuronal Death in Parkinson's Disease

Abstract: Oxidative stress is a major factor in Parkinson's Disease (PD). Dopamine (DA) itself is easily oxidized to quinone derivatives and reactive oxygen species (ROS) that impair energy metabolism and form adducts with proteins such as upsilon-synuclein. Because pharmacological depletion of DA in animal models is confounded by non-specific peripheral and central nervous system effects, the role of DA oxidation in nigral cell death has been previously impossible to address. Thus a key unanswered hypothesis in this field is that DA oxidation is a major contributor to the death of dopaminergic neurons in PD. The proposed studies address several aspects of this hypothesis including the interaction of known environmental factors in triggering DA oxidation. Specifically, the hypothesis that the DA-releasing potential of the parkinsonism-inducing drug, MPP+, is due to its ability to exchange with DA and/or to reduce intracellular pH gradients will be addressed using newly derived mice expressing enhanced green fluorescent protein from a dopaminergic locus (TH+/eGFP). Primary cultures derived from these animals as well purified synaptosomal and vesicular preparations from dopaminergic terminal fields will be used in combination with fluorescent and radioactive probes to determine the temporal aspects of DA release, intracellular membrane changes, ROS formation, ATP loss, etc in response to toxin treatment. In addition, the hypothesis that DA oxidation contributes to the death of dopaminergic cells will be directly tested in vivo using animals genetically engineered to have different levels of DA production. Behavioral, oxidative and immunocytochemical criteria will be used to establish the role of DA in both the acute and chronic MPTP model of PD. To test whether DA depletion prevents ROS, new methodologies to detect in situ ROS will be used with a battery of antibodies directed against nitrotyrosine, nitrated alpha-synuclein, etc. to temporally evaluate ROS formation following acute or chronic MPTP administration in DA deficient and wild type animals. Taken together, the proposed studies will determine whether DA oxidation plays a central role in the death of DA synthesizing cells and provide insights impossible to obtain from standard animal models. Knowledge of the source and cascade of events surrounding DA-induced free radical formation will help answer risk-benefit controversies surrounding the use of dopamine replacement therapies as well as facilitate the development of new drugs and/or treatment strategies in the pathogenesis of PD. -

Principal Investigator: PAPA, STELLA M
Grant Number: 1R01NS045962-01A1
Title: Regulation of Motor Function in Parkinson's Disease

Abstract: Motor disturbances of Parkinson's disease are caused by a series of functional alterations in the basal ganglia that derive from dopamine denervation. The mechanisms underlying those functional alterations are not completely understood yet. Moreover, long-term levodopa therapy is usually associated with disabling motor complications, such as motor fluctuations and dyskinesias, whose pathophysiology also remains obscure. The long-term objective of this project is to elucidate the pathophysiologic mechanisms of abnormal motor behaviors in Parkinson's disease in view of developing new and specific therapeutic tools. Thus, this study is aimed: -firstly, to localize functional alterations in specific basal ganglia circuits; -secondly, to determine the glutamate regulation associated to an altered neuronal function; -finally, and based on the foregoing data, to explore new therapeutic approaches by interacting with the glutamatergic neurotransmission in a region-specific manner. Specifically this project comprises three aims: 1. To study the neuronal activity of individual basal ganglia regions by single cell recording in normal and various groups of parkinsonian monkeys (MPTP-treated primates) that exhibit different motor behaviors depending on treatment conditions (i.e.: parkinsonian state, its normalization, and drug-induced dyskinesias). 2. To study the glutamate receptor sensitivity in basal ganglia regions in relation to different motor conditions by comparing the binding of receptors across animal groups. 3. To study the glutamatergic blockade in restricted basal ganglia regions by determining its effects on neuronal activity and motor behavior. The research design includes techniques ranging from single- and multiple single- unit recording of neuronal activity, autoradiographic binding of receptors, to intracerebral administration of drugs in parkinsonian monkeys whose motor abnormalities closely resemble the human disease. This project proposes a novel approach to a comprehensive study of the abnormal motor function in Parkinson's disease. Thus, it will largely contribute to the rationale for new treatments that selectively target particular motor conditions. -

Principal Investigator: PARNG, CHUENLEI
Grant Number: 1R43NS048607-01
Title: In Vivo Screen for Neuroprotective Agents

Abstract: Aberrant apoptosis is implicated in several neurodegenerative disorders including, stroke, brain trauma, spinal cord injury, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Alzheimer's and Huntington's disease. These neurodegenerative diseases are associated with high morbidity and mortality, and treatment options are limited. Agents that modulate apoptosis are a major focus of drug development efforts by biopharmaceutical companies. Assessment of drug effects in a convenient vertebrate model, prior to proceeding to evaluation in complex systems, such as mouse, can potentially streamline drug development and dramatically reduce costs. Zebrafish mutants exhibiting aberrant apoptosis in the central nervous system are an excellent animal model for studying neurodegeneration. Using a zebrafish neurodegenerative mutant line and a vital dye apoptosis assay, this Small Business Innovation Research project proposes to characterize embryogenesis and apoptotic patterning in zebrafish embryos, and to develop a rapid and effective in vivo screen for neuroprotective therapeutics.-

Principal Investigator: POWERS, WILLIAM J
Grant Number: 5R01NS041771-04
Title: CEREBRAL MITOCHONDRIAL METABOLISM IN NEURODEGENERATION

Abstract: Several lines of evidence suggest that Huntington's disease (HD) and Parkinson's disease (PD) have defects in mitochondrial function that impair oxidative phosphorylation and play a key role in the mechanism of neuronal death. To date, however, there have been no direct measurements of cerebral oxygen to glucose metabolic ratios to demonstrate an in vivo defect in cerebral mitochondrial metabolism in these diseases. We will use positron emission tomography (PET) to measure in vivo regional cerebral oxygen metabolism (CMR02) and cerebral glucose metabolism (CMRglc) to test two primary hypotheses: 1) Patients with HD have a generalized defect in cerebral mitochondrial metabolism. To test this hypothesis, we will measure whole brain CMR02/CMRgIc in 15 gene-positive pre-symptomatic patients with HD, 15 gene-positive patients with HD and definite motor signs and 30 age/gender-matched normal controls. 2) Patients with PD have a generalized defect in cerebral mitochondrial metabolism. To test this hypothesis, we will measure whole brain CMR02/CMRgIc in 15 never-medicated, early PD patients and 15 age/gender-matched normal controls. In the same subjects, we also will test two secondary hypotheses: 3) Regions vulnerable to pathologic insult have larger magnitude or selective defects in cerebral mitochodrial metabolism - caudate and putamen in HD and substantia nigra and putamen in PD. 4) In PD and HD, the degree of dysfunction in platelet electron transport complex function measured in vitro correlates with the degree of abnormal cerebral mitochondrial metabolism measured in vivo. At this time it is not clear how the abnormalities in electron transport chain activity measured in vitro in these two diseases correspond to cerebral mitochondrial metabolism in vivo. Direct in vivo regional PET measurements of CMR02 and CMRglc will allow us to demonstrate the extent and magnitude of mitochondrial dysfunction in vivo. Establishing the existence of cerebral mitochondrial dysfunction early in the course of these diseases will not only provide insights into the pathogenesis, but it will provide a measurable biological abnormality that can be monitored to determine the effect of treatments aimed at slowing or halting the progression of neuronal loss. The opportunity to determine the relation between platelet mitochondrial function and cerebral mitochondrial metabolism in patients with PD and HD is uniquely important. If such a relationship can be established in untreated patients in this study, then we would pursue further studies to determine the effects on cerebral mitochondrial metabolism of agents that alter platelet mitochondrial function. If such studies yield consistent results, they will establish the basis for

Principal Investigator: QUIK, MARYKA
Grant Number: 5R01NS042091-02
Title: Nicotinic Receptors in Parkinson's Disease

Abstract: Our goal is to determine the role of nicotinic receptor subtypes in the basal ganglia with the long-term objective of developing novel therapeutic strategies for Parkinson's disease. The rationale for this work is based on studies showing that nicotine administration improves locomotor deficits after a nigrostriatal lesion and, furthermore, that nicotine or smoking results in an apparent protective effect against nigrostriatal damage in rodents and in Parkinson's disease. However, multiple nicotinic receptors are activated by nicotine to result in beneficial but also side effects in both the peripheral and central nervous system. We hypothesize that specific nicotinic receptor populations in the brain are involved based on work demonstrating that the distinct subtypes have unique localization and molecular functions. We will approach these studies through four specific aims. As a crucial first step, we will identify the regional and cellular localization of nicotinic receptor subtypes in basal ganglia and determine the changes in receptor subtypes after nigrostriatal degeneration. This will be done using three different approaches including in situ hybridization, receptor autoradiography and immunocytochemistry and form the basis of Specific Aims 1 and 2. We will then initiate studies to assess the nicotinic receptor subtypes involved in striatal function as described in Specific Aim 3, followed by behavioral studies (Specific Aim 4) to determine the ability of nicotinic agonists to reverse parkinsonism. This work should increase our understanding of the role of different nicotinic receptor subtypes in basal ganglia function. In summary, the proposed work should provide new insight concerning alterations in nicotinic receptor subtypes after nigrostriatal damage and nicotinic agonist treatment. These data together with the results of the behavioral studies may form a basis for the use of nicotinic drugs in the treatment of Parkinson's disease. -

Principal Investigator: QUIK, MARYKA
Grant Number: 1R01NS047162-01
Title: Nicotinic and neuroprotection in a parkinson mouse model

Abstract: Our goal is to understand the effects of nigrostriatal damage and nicotine treatment on nicotinic receptor (nAChR) subtypes in the basal ganglia, and determine the relationship of any changes to neuroprotection. The rationale for such work is based, in part, on epidemiological studies showing that there is a decreased incidence of Parkinson's disease (PD) in smokers. This apparent neuroprotection may be due to nicotine in tobacco since nicotine protects against nigrostriatal damage in various experimental models. Nicotine exerts its effects by stimulating nAChRs. We hypothesize that nicotine-mediated protection against nigrostriatal damage occurs as a consequence of changes in nAChR subtypes. Our preliminary data show that there are differential changes in nAChRs subtypes and their function after MPTP treatment. In this proposal, we will test the effects of nicotine to modulate nAChRs, study its neuroprotective effects against nigrostriatal degeneration and investigate its mechanism(s) of action. This will be approached through the following Specific Aims. (1) We will test the hypothesis that nicotine administration influences nAChR expression and function in MPTP-treated mice. Although nicotine exposure is well-known to upregulate nAChRs in control animals, studies to determine its effects after nigrostriatal damage remain to be done. Next (2) we will test the hypothesis that nicotine-induced changes in nAChRs correlate with neuroprotection against nigrostriatal damage by measuring various markers of striatal dopaminergic function. These data will be correlated to changes in nAChRs to determine whether receptor alterations are linked to neuroprotection. (3) To determine whether specific nicotinic receptor subtypes are involved we will we will study whether nicotine protects against nigrostriatal damage in nAChR knockout mice. (4) Finally, experiments will be done to study the molecular mechanisms that mediate nicotine-induced neuroprotection. We will investigate the hypothesis that trophic factors such as basic fibroblast growth factor (bFGF) and brain derived neurotrophic factor (BDNF), as well as immune mediators such as interleukin-6, are involved. These studies will enhance our knowledge of the changes in nAChR expression and function with chronic nigrostriatal damage and nicotine treatment. This may allow for the design of neuroprotective strategies for PD, a disorder for which only symptomatic treatment is currently available.-

Principal Investigator: RACETTE, BRAD A
Grant Number: 3U10NS044455-02S1
Title: PARKINSON DISEASE NEUROPROTECTIVE TRIAL:CLINICAL CENTER

Abstract: Unavailable

Principal Investigator: RATAN, RAJIV R
Grant Number: 5R01NS040591-04
Title: Arginase and Regulation of Nitric Oxide Synthase in ALS

Abstract: Amyotrophic lateral sclerosis is a prevalent neurological disorder characterized by inexorable muscle weakness leading to death. The principal pathological finding in amyotrophic lateral sclerosis is loss of nerve cells in the anterior horns of the spinal cord, the motor nuclei of the brainstem, and the upper motor neurons of the cerebral cortex. Investigations aimed at preventing or limiting progression of amyotrophic lateral sclerosis have thus focused on the mechanisms by which neurons degenerate. A transgenic mouse model has been developed that possesses many of the pathological and clinical features of human familial and sporadic amyotrophic lateral sclerosis. As nitric oxide (NO) has been shown to mediate neuronal loss in other neurodegenerative conditions, several groups have investigated the role that NO may play in disease progression

Principal Investigator: REDMOND, D EUGENE
Grant Number: 1U01NS046028-01A1
Title: GDNF Delivery to MPTP Monkeys by EIAV lentivirus and AAV

Abstract: An effective gene therapy for Parkinson's disease is the goal of this proposal, which will test the effectiveness and safety of human glial cell line derived neurotrophic factor (GDNF) delivered by two improved vector systems derived from equine infectious anemia virus (EIAV) or from adenoassociated virus (AAV). Both vectors deliver the cellular marker gene, nuclear localized lacZ (lacZnl) or GDNF efficiently and stably into nigrostriatal target regions, can be regulated using a tetracycline promoter system, and offer additional safety that the respective wild-type viruses do not cause any disease in humans. The recombinant vectors will be tested in the parkinsonian model produced by the neurotoxin MPTP in monkeys. GDNF has shown promise for preventing or reversing morphological, biochemical and functional deficits in other models of Parkinson's disease in rodents and primates, using rAAV, and rHIV. But these studies also showed important problems to be solved to ensure that a GDNF gene therapy will be safe and effective in patients. Concerns about inflammatory, cytotoxic, inadequate or excessive gene expression, persistence, viral recombination or replication have led to the development of improved and safer vectors with regulatable promoters, which will be tested in this proposed project. Initial studies will address transgene expression (lacZnl or GDNF) in normal African green monkeys, determining effective titers, transduction efficiency, cellular tropism, distribution, level, and stability of transgene expression, neuropathology and host cellular responses after delivery by rEIAV or rAAV. Each of the two vectors will then be used to deliver GDNF to the nigrostriatal system of MPTP parkinsonian monkeys to test hypotheses that GDNF expression will improve function in both moderate and severely parkinsonian monkeys for periods up to 24 months. The most effective procedures will be optimized by comparing injection sites, a regulatable promoter to inactivate gene expression, and safety of all procedures including high injection titers. Measures of efficacy will include behavioral parameters, molecular assays of transgene expression using ELISA for protein, RT-PCR for mRNA and PCR for vector DNA, biochemical assays of DA and its metabolites, neuroanatomical and morphometric analyses, neuropathology, clinical chemistry, SPECT imaging, and autoradiography. These studies aim to provide the necessary data to initiate successful clinical trials in Parkinson's patients at the earliest possible time. -

Principal Investigator: RODRIGUEZ, ALICE L
Grant Number: 1F32NS049865-01
Title: Development of allosteric potentiators of mGluR4

Abstract: Treatment of Parkinson's disease (PD) has traditionally focused on dopamine replacement strategies such as LDOPA. While generally effective early on, L-DOPA has often proven inadequate for long term treatment due to serious adverse side effects. Recent studies in Dr. Conn's laboratory suggest that activators of metabotropic glutamate receptor mGluR4 may provide a novel pharmacological approach to the treatment of PD by targeting the indirect pathway of the basal ganglia. Furthermore, Dr. Conn and coworkers have developed a novel approach to activation of mGluR4 by development of allosteric potentiators that do not activate this receptor directly but dramatically potentiate the response to glutamate. While these studies provide an exciting proof of principle for a novel approach to activation of mGluR4, there is a need to develop novel compounds that have a higher potency and are useful for further in vivo studies. The goal of this work is to develop novel potent and selective allosteric potentiators of mGluR4. A threefold approach will be implemented, beginning with performing a high throughput screen mining for compounds that potentiate the glutamate response of mGluR4. In parallel with the HTS, medicinal chemistry studies will be pursued to improve upon the properties of known potentiators. Finally, mutagenesis studies will be performed to develop a better understanding of the molecular interactions involved in potentiator binding which will subsequently aid in the design of future compounds. Together these approaches will result in the development of novel small molecules that have a therapeutic effect on PD by reducing transmission through the indirect pathway. Furthermore, these studies will be complemented by ongoing electrophysiology and behavioral studies in Dr. Conn's laboratory that will determine the effects of these compounds in vitro models of basal ganglia function. -

Principal Investigator: ROSS, GEORGE WEBSTER
Grant Number: 3U10NS044448-02S1
Title: Parkinson's Disease Neuroprotection Trial: Hawaii Center

Abstract: Unavailable

Principal Investigator: RUOHO, ARNOLD E
Grant Number: 5R01NS033650-09
Title: Characterization of Vesicular Monoamine Transporters

Abstract: The strategy of this proposal is based on the rationale that identification of the inhibitor, substrate, proton translocation, and functionally relevant phosphorylation sites on monoamine transporters (VMAT2) will provide a basic understanding of the mechanism of action of monoamine sequestration into vesicles and the factors which regulate transporter activity. This work will be accomplished in three Specific Aims: (1) Identification of the reserpine binding site(s) on VMAT2. Novel reserpine photoaffinity labels will be synthesized and characterized, and photo-labelled peptides will be identified in order to map the reserpine binding site; (2) Identification of the substrate transport channel. This aim will involve the use of several approaches, including radioactive photo-activatable substrate analogs to covalently derivatize the substrate binding site on VMAT2; site-specific derivatization of VMAT2 at engineered cysteine residues with the cysteine-reactive reagents, methanethiosulfonate ethyl amine (MTSEA), and MTS-ethyltrimethylammonium (MTSET); and site-directed mutagenesis of potential residues lining the channel; (3) Determination of the functional role of two highly charged regions of VMAT2. This aim will involve the use of biochemical and genetic (site-directed mutagenesis) approaches to determine the role of phosphorylation of the N-terminus of VMAT2 on transporter function and the intracellular distribution/oligomeric state of the transporter. Reduced or aberrant activity of the monoamine transporter of the synaptic vesicles in dopaminergic neurons of the substantia nigra through either direct or indirect actions of toxicants (e.g., MPP+, insecticides) and genetically altered neuronally expressed proteins may play a central role in Parkinson's Disease. The regulation of uptake of monoamine neurotransmitters into storage vesicles may also play an important role in affective psychological disorders related to depression by altering levels of serotonin, norepinephrine, dopamine, or other neurotransmitters. This work will provide insight into the mechanism of action of the monoamine transporters and contribute to our understanding of how pharmacological and therapeutic strategies may be devised to treat Parkinsonism or other disorders of the nervous system. -

Principal Investigator: SAGE, JACOB I
Grant Number: 3U10NS044415-02S1
Title: Parkinson's Disease Neuroprotection Clinical Trial

Abstract: Unavailable

Principal Investigator: SALAMONE, JOHN
Grant Number: 1R01NS047261-01
Title: Dopamine D2 and Adenosine A2A roles:Tremulous Movements

Abstract: Symptoms of parkinsonism, such as akinesia, bradykinesia, and tremor, can be caused by degeneration of dopamine (DA) neurons, or by administration of DA antagonist drugs. Parkinsonism is characterized by a cascade of neurochemical events that reflect interactions between several neurotransmitters in the circuitry of the basal ganglia, including DA, acetylcholine, serotonin, GABA and adenosine. Within the last few years, increasing evidence has accumulated indicating that central adenosine neurons play an important role in modulating the functional circuitry of the basal ganglia. Several subtypes of adenosine receptors are involved in motor function, and anatomical studies have demonstrated that the adensonine A2A receptor subtype has a relatively high degree of expression within the striatum. Although several types of striatal cells contain some adensonine A2A receptors, these receptors are present in very high densities on striatopallidal neurons, which also tend to co-express DA D2 receptors and enkephalin. It has been suggested that antagonists of adenosine A2A receptors could have some potential utility as antiparkinsonian drugs. In a recent study from our laboratory, it was demonstrated that IP injections of the adenosine A2A antagonist, KF17837, also suppressed haloperidol-induced tremulous jaw movements, and reversed the locomotor suppression induced by this D2 antagonist. This profile of activity is consistent with the hypothesis that antagonism of adenosine A2A receptors can result in antiparkinsonian effects in animal models. The proposed experiments are designed to investigate the role of the striatopallidal GABAergic pathway as a possible mediator of the putative antiparkinsonian effects of adenosine A2A antagonists. These proposed studies will focus on the tremulous jaw movement model, which is related to parkinsonian tremor. It is hypothesized that adenosine A2A antagonists are acting on striatopallidal GABAergic neurons that also express DA D2 receptors. In view of research showing that haloperidol increases extracellular GABA in globus pallidus, and that haloperidol-induced tremulous jaw movements are reduced by pallidal injections of bicuculline, it is hypothesized that doses of adenosine A2A antagonists that reduce jaw movement activity also will reduce haloperidol-induced increases in GABA release in globus pallidus. In addition, it is hypothesized that adenosine agonists and antagonists will interact to regulate the behavioral and neurochemical effects of haloperidol. These hypotheses will be investigated using studies that involve both systemic and intrastriatal injections of drugs that act upon A2A receptors, and the proposed work will involve a

Principal Investigator: SARANG, SATINDER S
Grant Number: 1R43NS050920-01
Title: PESTICIDE-SYNUCLEIN INTERACTIONS AS RISK FACTORS FOR PD

Abstract: Parkinson's disease (PD) and other age-associated neurological disorders represent one of the largest unmet medical needs in developed countries. However, the discovery of improved diagnostics and therapeutics for these disorders is hampered by incomplete understanding of underlying disease mechanisms and risk factors. Oxidative stress, mitochondrial dysfunction, and protein aggregation have been implicated as major mechanisms causing dopaminergic neuronal loss in PD. Epidemiological studies have revealed an association between pesticide exposure and PD, and pesticides that cause oxidative stress and mitochondrial dysfunction, such as rotenone and paraquat, are used in cellular and animal models of PD. Furthermore, interactions between pesticides and the PD-linked gene alpha-synuclein have been postulated. Although almost 1000 pesticide active ingredients are currently marketed, these compounds have not been systematically screened for neurotoxicity in cellular or animal models of PD. The identification of pesticides that interact with alpha-synuclein to cause neurodegeneration may lead to the discovery of novel candidate risk factors and more representative disease models for PD. For this proposal, investigators at Cambria Biosciences will exploit a published moderate-to-high throughput neuronal cell-based model of PD, with the goal of identifying individual pesticides and synergistic pesticide combinations potentially involved in the pathogenesis of PD. Our established cellbased model of PD will be used to screen -approximately 350 registered pesticides to identify neurotoxic pesticides. Our specific aims include: (1a) identifying neurally-active pesticides that induce cell injury to two PD-like cell lines that stably express wild type (WT) human alpha-synuclein and mutant A53T alpha-synuclein; (1b) identifying any synergistic effects of neurotoxic pesticides in inducing cell damage in these a-synuclein-expressing neuronal cells; and (2) characterizing the activity of these neurotoxic pesticides and pesticide combinations using primary mature mesencephalic DA neurons. The identified neurotoxic pesticides will be employed in follow-on Phase II studies for the development of improved in vitro and in vivo PD models, which will ultimately be used to screen for neuroprotective compounds as part of a comprehensive drug discovery program. -

Principal Investigator: SCHNEIDER, JAY S
Grant Number: 2R01NS038681-06
Title: GM1 Ganglioside Effects on Parkinson's Disease

Abstract: Parkinson's disease (PD) is a slowly but relentlessly progressive neurodegenerative disorder resulting in a time-dependent worsening of clinical symptoms. No drug has yet been identified that definitively slows or stops the progression of PD or substantially forestalls the inevitable functional decline in PD patients. Thus, disease modifying drugs that can modify clinical progression, enhance repair of damaged neurons, remediate existing neuropathological deficits, restore or enhance function of residual parts of the dopamine (DA) system and/or activate compensatory mechanisms are sorely needed. GM1 ganglioside may be such a treatment. In vitro and in vivo studies have shown GM1 to rescue damaged DA neurons, stimulate survival and repair of DAergic neuron and sprouting of functional DAergic terminals, increase DA levels in the striatum and upregulate DA synthetic capacity of residual neurons. Preliminary clinical studies of GM1 in PD patients have shown clinical improvements in patients with short-term use of GM1 and minimal symptom progression in patients with 2 to 5 years of GM1 use with resumed progression of symptoms following discontinuation of long-term GM1 use. The specific aims of this research are: 1) Assess the clinical efficacy of GM1 and the relationship between clinical improvement and in vivo quantitation of the integrity of the striatal DAergic innervation (assessed by PET imaging of the dopamine transporter site) in patients with typical mild/moderate PD in a randomized double blind placebo-controlled clinical trial. Working hypothesis: GM1 ganglioside treatment will result in symptomatic improvements related to effects on damaged but viable DA neurons and this may be accomplished through sprouting of functional DAergic terminals in the striatum. 2) Assess the extent to which long-term (2 years) use of GM1 ganglioside may stabilize symptoms or slow symptom/disease progression in PD patients (using clinical evaluations and PET imaging of the dopamine transporter as a surrogate measure). Working hypothesis: Long-term GM1 use will stabilize symptoms or slow the progression of symptoms in PD patients and this may be accompanied by reduced loss of striatal DA terminals over time. -

Principal Investigator: SCHOR, NINA F
Grant Number: 5R01NS041297-03
Title: Antioxidant Strategies for Parkinson's Disease

Abstract: Reactive oxygen species (ROS) have been implicated in the pathogenesis of Parkinson's disease. This suggests that antioxidant strategies may be useful in the treatment and/or prevention of this neurodegenerative disorder. We have developed and implemented two models for the central movement disorder and autonomic peripheral neuropathy, respectively, associated with Parkinson's disease. We propose to use these models to design and test antioxidant strategies we have previously developed for adjunctive use with ROS-generating chemotherapeutic agents. We will further use our studies of the biochemical effects of antioxidant treatment to develop a screening test for new antioxidant agents for use in Parkinson's disease and other ROS-related disorders. Specifically, we propose to test the hypothesis that recycling antioxidants increase expression of p21 wafl/cip1,enhance binding of HIF-1 and CREB to DNA, activate NF-kappaB, prevent ROS-induced morphological apoptosis, and decrease ROS-induced membrane phospholipid and protein nitration in culture models of Parkinson's disease. We will further test recycling antioxidants for their distribution to the CNS and peripheral compartments, and use this information to test CNS-penetrating and non-CNS-penetrating agents for efficacy in the central and autonomic nervous system models, respectively, of Parkinson's disease. Finally, we will test the hypothesis that the magnitude of induced in vitro biochemical change for each drug correlates with the degree of protection from the effects of ROS in the CNS or autonomic model. This latter study will pave the way for development of an in vitro screening test for new antioxidant strategies proposed for use in Parkinson's disease. This application specifically addresses the NINDS agenda for research in Parkinson's disease in its development of in vitro screening tests for putative therapeutic agents in general and antioxidants in particular for this disease, its development of animal models for the clinical aspects of Parkinson's disease, and its potential for further elucidation of the mechanisms of ROS-induced apoptosis in the nervous system.-

Principal Investigator: Sheline, CHristian T
Grant Number: 5R01NS030337-13
Title: ZINC NEUROTOXICITY

Abstract: Glutamate receptor- and Ca2+-mediated neurotoxicity was the focus of study during past grant periods. Recently, we have begun to examine a related form of neurotoxicity, also enhanced by glutamate receptor activation but mediated by Zn2+ rather than Ca2+. Zn2+-mediated neurotoxicity likely contributes to central neuronal death after certain insults, such as transient global ischemia. Our Central Hypothesis is that extracellular Zn2+ can kill neurons by: 1) entering across the plasma membrane, largely through voltage-gated Ca2+ channels (VGCCs) in depolarized neurons; 2) increasing intracellular free Zn2+ ([Zn2+]i); 3) interfering with glycolysis, causing ATP levels to fall; 4) triggering apoptosis (at lower Zn2+ levels). The proposed experiments will test aspects of this central hypothesis in cultured murine cortical neurons, delineating mechanisms underlying Zn2+-induced neuronal death to advance efforts to develop therapeutic countermeasures that might be used to reduce brain damage after cardiac arrest. Cultured neurons will be exposed to varying concentrations of extracellular zinc for brief ("fast toxicity") or prolonged ("slow toxicity") time periods. We plan to define the relationships linking transmembrane Zn2+ influx (measured with patch-clamp and radio-isotope flux techniques), [Zn2+]I (measured with dye videomacroscopy), cellular Zn2+ content (measured with atomic absorption spectroscopy or inductively-coupled plasma spectroscopy), and cellular apoptosis (v.s. necrosis). We will also measure resultant neuronal levels of ATP, NAD+, NADH and glycolytic intermediates, mitochondrial transmembrane potential, and cytoplasmic reactive oxygen species (measured with dihydroethidium dye). Finally, we will test genetic perturbations of cellular Zn2+ homeostasis, specifically increased or decreased expression of the key plasma membrane Zn2+ transporter, ZnT-1, or the major neuronal intracellular Zn2+ binding protein, metallothionein-III, will produce the changes in vulnerability to Zn2+ neurotoxicity predicted by the central hypothesis. -

Principal Investigator: Sibley, David
Grant Number: 5Z01NS002263-28
Title: Molecular And Pharmacological Studies Of Dopamine Receptors

Abstract: Unavailable

Principal Investigator: SILVERMAN, RICHARD B
Grant Number: 1R01NS047331-01A1
Title: Celestrols for Treatment of Neurodegenerative Diseases

Abstract: The expression of molecular chaperones has been shown to suppress protein misfolding/aggregation and cellular toxicity phenotypes in model systems associated with Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, and ALS. A feature common to diseases of protein conformation is the appearance of folded intermediates that self-associate to form protein aggregates and inclusions. The molecular chaperones Hsp90 and Hsp70 sequester damaged proteins that appear in cells exposed to physiological and environmental stress. The ability of molecular chaperones to suppress the cellular toxicities associated with expression of these "toxic" proteins may be due to the intrinsic properties of chaperones to capture and suppress the appearance of folded intermediates. Therefore, we propose that the identification of small molecules that elevate the expression of genes encoding heat shock proteins and molecular chaperones should lead to the development of novel therapies beneficial to the prevention of neurodegenerative diseases. The rationale for this proposal is based on results obtained by our laboratory and others who participated recently in a screening program organized by the NINDS, Huntington Disease Society of America, Hereditary Disease Foundation, and the ALSA to identify new drugs for treating these diseases. A search was carried out for drugs that activate the heat shock response; the most effective compound identified was the natural product celastrol. Synthetic analogs of celastrol will be prepared to optimize its effectiveness as a regulator of the heat shock response and a suppressor of neurotoxicity and to determine its mechanism of action as an activator of the heat shock response. To probe the function of celastrol as a potential therapy for neurodegenerative diseases, the following Specific Aims will be addressed: (1) Synthesize analogs of celastrol that induce the human heat shock response using a heat shock promoter-reporter assay in human tissue culture cells. (2) Determine the mechanism of action of celastrol (or an analog). The working model is that celastrol activates the heat shock response by inducing heat shock transcription factor HSF1. The mechanism by which HSF1 activity is induced by celastrol will be determined. It also will be determined whether celastrol, by virtue of its ability to activate the expression of chaperones, can reduce the aggregation and neurotoxicity of the Huntington Q64 protein expressed in a human SH-SY5Y neuroblastoma cell line. (3) Studies will be carried out to identify the binding target for celastrol using molecular biological and biochemical techniques. Identified target(s) will then be cloned and characterized. Results of these studies

Principal Investigator: SMITH, YOLAND
Grant Number: 5R01NS042937-03
Title: GABA-B RECEPTORS AND PARKINSON'S DISEASE

Abstract: Three major receptor subtypes mediate GABAergic inhibitory effects in the mammalian CNS, the GABA-A and GABA-C receptors that generate fast inhibition, and the metabotropic GABA-B receptors (GBR1, GBR2) which mediate slow inhibitory effects via activation of an intracellular second messengers cascade. Data from our laboratory showed that GBR1 receptors are strongly expressed pre- and postsynaptically throughout the monkey basal ganglia. Interestingly, pre-synaptic GBR1 immunoreactivity is mainly associated with glutamatergic terminals suggesting that GABA-B receptors act as heteroreceptors that modulate glutamate release in these structures. To further elucidate the roles of GABA-B receptors in basal ganglia, we propose a series of anatomical, neurochemical and behavioral studies to characterize various aspects of GABA-B receptor localization and functions in the globus pallidus (GP) and subthalamic nucleus (STN) of normal and parkinsonian monkeys. It is well established that overactivity of glutamatergic pathways from the STN to basal ganglia output structures, namely the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr), is a cardinal feature of the pathophysiology of Parkinson's disease (PD). Our preliminary data raise the interesting possibility that activation of presynaptic GABA-B receptors in GP and STN may reduce transmission at overactive subthalamofugal synapses. In this model, activation of GABA-B receptors would attenuate some of the parkinsonian motor symptoms. In support of this notion, our data also indicate that local administration of GABA-B receptor agonists in GPi and STN reduces glutamate release in primates and that systemic application of these compounds may have beneficial therapeutic effects in parkinsonian monkeys. The following four specific aims are proposed: (1) Characterize and compare the pattern of subcellular and subsynaptic localization of GABA-B Ri immunoreactivity in the GP and STh of normal and parkinsonian monkeys, (2) Determine the exact source of glutamatergic axon terminals that express pre-synaptic GABA-B receptors in GP and STN, (3) Test the possibility that local application of GABA-B agonists decreases glutamate levels in the GP and STN of normal monkeys and (4) Test the potential therapeutic effects of GABA-B receptor agonists in parkinsonian monkeys. These experiments will further delineate the subsynaptic localization and roles of GABA-B receptors in modulating glutamate release and open novel research avenues for the potential use of GABA-B agonists in the pharmacotherapy of PD. -

Principal Investigator: SOGHOMONIAN, JEAN-
Grant Number: 5R01NS040783-03
Title: Behavioral Sensitization and Parkinson's Disease

Abstract: The systemic administration of agonists of dopamine receptors remains one of the most effective therapeutic interventions used for the symptomatic treatment of Parkinson's disease. However, the chronic administration of these agonists over several months-years can induce the gradual development of debilitating abnormal involuntary movements such as dyskinesia. Current models of the basal ganglia favor the hypothesis that the chronic administration of dopaminergic agents involves an increased/abnormal GABA signaling in the substantia nigra, pars reticulata (SNr), and in the internal pallidum. We propose to examine this hypothesis in a rodent experimental model of Parkinson's disease. The specific aims are: 1-To test the hypothesis that the chronic administration of agonists of dopamine receptors to 6-OHDA-lesioned rats alters the expression of molecules involved in the regulation of GABA levels in neurons that provide an input to the SNr/internal pallidum; 2-To test the hypothesis that increases in basal extracellular GABA levels in the SNr/internal pallidum are involved in the effects of long-term administration of agonists. 3-To test the hypothesis that plasticity of GABA receptors in the SNr plays a role in the effects of chronic administration of dopaminergic agents. These studies will involve quantitative in situ hybridization histochemistry to measure changes in mRNA levels, microdialysis to measure changes in GABA levels and intranigral administration of pharmacological agents acting on GABA levels or GABA receptors to alter agonist-induced circling in rats unilaterally lesioned with 6-OHDA. -

Principal Investigator: STANDAERT, DAVID G
Grant Number: 5R01NS034361-09
Title: NMDA RECEPTORS--REGULATION OF BASAL GANGLIA FUNCTION

Abstract: Glutamate is the principal excitatory neurotransmitter in the brain and has an important role in the regulation of movement. N-methyl-D-aspartate (NMDA) glutamate receptors are of particular interest because they are involved in long-term processes such as neural adaptation and memory. Drugs acting at NMDA receptors have important therapeutic potential in human Parkinson's disease. In particular, recent work has suggested that changes in NMDA receptor properties may be responsible for the development of the motor complications of levodopa therapy, such as wearing off and dyskinesias. Such motor complications occur in the majority of patients with Parkinson's disease and are frequently the principal cause of disability. NMDA receptors are assembled from proteins from two gene families, and receptors with different composition have distinct properties. The functions of the receptors are further regulated by differential trafficking and phosphorylation. Our investigations have revealed that neurons which serve different functions in the circuitry of the basal ganglia express different types of NMDA receptor subunits. In models of Parkinsonism, striatal NMDA receptors are modified. The most significant changes are not alterations in the level of gene expression, but rather are changes in the assembly, phosphorylation, and synaptic localization of the protein subunits. In this project, we will employ a variety of techniques to establish the nature and mechanisms of the modifications of basal ganglia NMDA receptors produced by chronic dopamine depletion and dopamine replacement therapy. The long term goal of this work is to gain insight into the cause of wearing off and dyskinesias in Parkinson's disease, and develop better treatments for this common and disabling neurological disorder. -

Principal Investigator: STERN, CHANTAL E.
Grant Number: 5R01NS041636-04
Title: Cognitive, Pharmacological and fMRI Studies of Memory

Abstract: The proposed experiments will use cognitive and pharmacological manipulations coupled with functional MRI (fMRI) to test the modulatory regulation of memory formation. The experiments will test the hypothesis that acetylcholine enhances the sensory activation of cortical regions, and decreases interference from previously stored information during long term encoding and working memory. In Experiment #1, subjects will perform an encoding task using sequential lists of complex images in the fMRI scanner before and after iv injections of the centrally acting muscarinic cholinergic antagonist scopolamine, or after injections of the peripherally selective muscarinic antagonist glycopyrrolate. We predict that scopolamine, but not glycopyrrolate, will prevent an increase in fMRI activation during episodic encoding, and will be associated with a decrease in list-specific recognition. In Experiment #2, subjects will perform a delayed match to sample task in the scanner with either novel or familiar stimuli before and after scopolamine. We predict that activation of medial temporal lobe (MTL) regions will be stronger for novel stimuli relative to activation of prefrontal cortex (PFC) which will be stronger for familiar stimuli. Effects of scopolamine on task performance will be associated with changes in prefrontal cortex activation for the familiar task and changes in medial temporal activation for the novel task. In experiment #3, subjects will perform a two-back working memory task before and after infections of scopolamine. We predict that delay period activation will be stronger in PFC relative to MTL for this task, and scopolamine will decrease delay period activity and match enhancement. Experiment #4 will localize regions activated by face and house stimuli before and after encoding of paired associations. We predict that learning of associations will enhance the co-activation of these two regions, and that scopolamine will enhance this co-activation and proactive interference from associations learned before injections of scopolamine. The use of pharmacological manipulations provides an opportunity to test hypotheses by experimentally manipulating circuit level mechanisms important for cognitive tasks. This research is particularly relevant for understanding the cortical dynamics induced by cholinergic modulation, This isrelevant to the pathological states of low cholinergic innervation in Alzheimer's disease and Lewy-body dementia, and the potential therapeutic efficacy of different agents, including acetyleholinesterase inhibitors and other agents on selective processes involved in human memory. -

Principal Investigator: Swanwick, CATHERINE
Grant Number: 5F31NS043831-03
Title: BDNF and Synaptic Plasticity in Levodopa Sensitization

Abstract: Currently the most effective treatment for Parkinson's disease (PD) is levodopa. However, for many patients the benefit of levodopa treatment is limited by the development of levodopa-induced dyskinesias over time. The proposed experiments test the principal hypothesis that BDNF induces levodopa sensitization in the 6-OHDA lesioned rat model of PD through modulation of striatal LTP. The experiments address two specific aims: 1) to demonstrate the existence of synaptic plasticity in the denervated striatum after levodopa sensitization and 2) to establish the role of BDNF as a modulator of this plasticity. For Specific Aim 1, synaptic efficacy will be measured in medium spiny neurons of the denervated striatum using evoked field potential recordings. The NMDA receptor antagonist APV will then be applied to test of this synaptic efficacy is NMDA receptor-dependent. For Specific Aim 2, in situ hybridization will be used to examine the expression of BDNF and its receptor TrkB in the striatum and the cerebral cortex of levodopa-sensitized rats. Evoked field potentials will then be measured both when exogenous BDNF is applied to unsensitized denervated striata and when TrkB-IgG fusion protein, a scavenger of endogenous BDFN, is applied to sensitized denervated striata.-

Principal Investigator: SZETO, HAZEL H
Grant Number: 1R21NS048295-01
Title: Cell-Permeable Peptides for Mitochondrial Protection

Abstract: The application is submitted in response to the Program Announcement (PAR-02-138) requesting applications for exploratory/developmental projects in translational research. This proposal seeks to identify candidate therapeutics for neurodegenerative disorders. We have recently discovered a small lipophilic cationic peptide DAPL (Dmt-D-Arg-Phe-Lys-NH2, where Dmt = 2', 6'-dimethyltyrosine) that is cell permeable and selectively targets mitochondria. Preliminary studies with isolated mouse liver mitochondria have shown that this small peptide can protect against mitochondrial permeability transition and swelling, and reduce accumulation of reactive oxygen species. By protecting against mitochondrial dysfunction, this peptide may potentially be useful in the treatment of numerous neurodegenerative disorders. We are now seeking short-term support to further explore the pharmacology of this lead peptide analog in protecting brain mitochondria against various mitochondrial toxins, and to discover new analogs of this peptide that might lead directly to a therapy development project for a particular neurological disorder. Our specific aims are as follows: 1) To examine the ability of DAPL to protect mitochondria dysfunction caused by calcium overloading, 3-nitropropionic acid (3NPA), 1-methyl-4-phenylpyridium ion (MPP+), and t-butyl hydroperoxide (tBHP; 2) To examine the ability of DAPL to protect against cell death caused by glutamate, 3NPA, MPP +, and tBHP; 3) To carry out structure-activity relationship (SAR) studies with DAPL analogs to identify the optimal peptide analog for further preclinical development. The results from these exploratory studies will guide us to the development of preclinical animal studies for evaluating the therapeutic potential of DAPL analogs in the treatment of stroke and various neurodegenerative disorders, including Parkinson's disease, Huntington's disease and Alzheimer's disease. Potential collaborators for the animal studies have already been identified.-

Principal Investigator: Tilley, Barbara C
Grant Number: 5U01NS043127-04
Title: Parkinson's Disease Clinical Trial: Statistical Center

Abstract: Unavailable

Principal Investigator: TILLEY, BARBARA C.
Grant Number: 3U01NS043127-04S1
Title: Parkinson's Disease Clinical Trial: Statistical Center

Abstract: Unavailable

Principal Investigator: WALKER, PAUL D
Grant Number: 5R01NS039013-04
Title: SEROTONIN CONTROL MECHANISMS OF BASAL GANGLIA FUNCTION

Abstract: Attempts to develop new and effective treatments for movement disorders such as Parkinson's disease have been hampered by an insufficient knowledge of how basal ganglia receptor systems adapt to the consequences of dopamine depletion. This research focuses on determining the role of upregulated serotonin 2A receptors, which we hypothesize provide a mechanism for serotonin to exert greater control over basal ganglia transmission and locomotor function under conditions of dopamine depletion. Our preliminary studies indicate that the target of the serotonin 2A receptor mechanism is the DIRECT striatonigral pathway which utilizes tachykinin neuropeptides colocalized with GABA. New experiments of this application will test the central hypothesis that: upregulated serotonin 2A receptor signaling provides a mechanism for serotonin to enhance striatonigral transmission under conditions of dopamine depletion which influences basal ganglia function and animal behavior. In Specific Aim 1, we will determine the functional consequences of an upregulated serotonin 2A receptor system on serotonin signal transduction within the dopamine depleted striatum by measuring serotonin 2A receptor binding, its linkage to phosphoinositol hydrolysis, its modulation of striatal membrane excitability, and its ability to trans-synaptically regulate striatal tachykinin and GABA expression. In Specific Aim 2, we will determine if tachykinin striatonigral neurons react to the stimulation of upregulated serotonin 2A receptors in the dopamine depleted animal by increasing tachykinin and GABA transmission in the substantia nigra. We will also study the impact of this regulation on locomotor behavior. Finally, in Specific Aim 3, we will determine how an upregulated serotonin 2A receptor system influences the ability of the striatonigral system to regulate basal ganglia dopamine and GABA metabolism, and how these systems influence behavioral recovery of the dopamine depleted animal. Information obtained from these studies will contribute to a better understanding of basal ganglia function and may change how serotonin pathways are considered when designing new pharmacological strategies for diseases which affect dopamine transmission. -

Principal Investigator: Walters, Judith
Grant Number: 5Z01NS002139-30
Title: Pharmacology And Physiology Of The Substantia Nigra And Basal Ganglia

Abstract: Unavailable

Principal Investigator: WATTS, RAY L
Grant Number: 3U10NS044547-03S1
Title: UAB PD Neuroprotection Clinical Trial Cent*

Abstract: Unavailable

Principal Investigator: WICHMANN, THOMAS N
Grant Number: 5R01NS042250-04
Title: Basal ganglia discharge patterns in parkinsonism

Abstract: The basal ganglia are part of larger circuit that involves thalamus and cortex. Cortical inputs reach striatum and subthalamic nucleus (STN), and are transmitted via internal pallidal segment (GPi) and substantia nigra pars reticulata (SNr) to influence the activity of thalamocortical neurons. The function of this circuitry is disturbed in Parkinson's disease because of loss of dopamine in the basal ganglia. Besides changes in discharge rates, basal ganglia neurons also develop significant abnormalities in their discharge patterns in parkinsonism. One of the most salient abnormalities is the appearance of synchronized oscillatory discharge in STN, the external pallidum (GPe), GPi/SNr, and frontal cortex (detected by EEG). Available data suggest that this may result from altered activity along the cortex-STN-GPi/SNrthalamocortical route. With a combination of extracellular basal ganglia recordings and EEG, the proposed primate experiments explore the relationship between oscillatory activity in cortex and basal ganglia and will test the hypothesis that oscillatory discharge in the cortex-basal ganglia circuitry contributes to parkinsonism. The correlation studies under specific aim (S.A.) 1 assess the link between neuronal discharge in the basal ganglia (GPe, STN GPi, SNr) and EEG with simultaneous recordings in both brain regions. The importance of striatal or extrastriatal dopamine loss for the development of oscillatory discharge in parkinsonism will be tested under S.A. 2 by studying changes in oscillatory activity in basal ganglia and cortex induced by microinjections of the dopamine receptor agonist apomorphine at striatal and extrastriatal basal ganglia sites in parkinsonian animals. The experiments under S.A. 3 will test whether blockade of glutamate receptors in STN (blocking corticosubthalamic inputs) reduces oscillatory activity in basal ganglia and cortex. Finally (S.A. 4), the hypothesis will be tested that synchronized oscillatory discharge in the basal ganglia, induced by electrical stimulation of STN with bursts of stimulation pulses at burst rates between 2 and 30 Hz, disrupts motor performance and induces parkinsonian motor abnormalities in normal monkeys. These studies will help to understand the significance of oscillatory discharge in the basal ganglia and cortex in parkinsonism. This may provide guidance in the development of drug treatments directed at normalizing abnormal discharge patterns, and may help to understand the mechanism of action of existing treatments for Parkinson's disease, including dopamine receptor agonists, glutamate receptor antagonists, and deep brain stimulators. -

Principal Investigator: Wilson, Charles J
Grant Number: 2R37NS037760-06
Title: Neostriatal Cholinergic Interneurons Firing Patterns

Abstract: Altered function of the neostriatal cholinergic interneurons has been implicated in the pathology of Parkinson's disease, Huntington's disease, and a variety of other disorders. The observation that cholinergic antagonists are clinically effective in treating Parkinson's disease has led many investigators to suggest that within the striatum there is a balance of opposing actions of dopamine and acetylcholine. Despite the explosion of information on the pharmacology of acetylcholine in the neostriatum, physiological information has been difficult to obtain due to the rarity of cholinergic interneurons compared to the other cells in the striatum. Using infrared differential interference contrast microscopy, we have recorded from identified cholinergic neurons in slices, and have shown that they are intrinsic pacemakers that exhibit three distinctly different spontaneous filing patterns, even in the absence of fast synaptic input (but with neuromodulators intact). One of the firing patterns resembles that seen in experimental Parkinsonism. This finding provides a window on several otherwise inexplicable observations, including the rhythmic synchronous activity of these neurons in monkeys rendered Parkinsonian by experimental treatment with MPTP. In the proposed experiments, we will employ whole ceil recording of identified cholinergic interneurons and calcium imaging in single cells to determine (1) The ionic mechanisms of the rhythmic bursting firing mode, which most resembles that seen in Parkinsonism, which we already know is related to modulation of calcium and calcium dependent ion channels (2) The basis for synchronization of cholinergic interneurons when they are firing in the bursting mode, including the synaptic connectivity among cholinergic cells and (3) The influence of D1 and D2 dopaminergic agonists and antagonists on the firing patterns of cholinergic interneurons. The effects of dopamine on firing pattern will be directly related to other studies on dopaminergic modulation of specific ion channels to provide an integrated understanding of the actions of dopamine on cholinergic interneurons and the neostriatal circuitry.-

Principal Investigator: WOOTEN, MARIE W
Grant Number: 5R21NS044847-02
Title: Development of Par4 Peptide for Treatment of Alzheimers

Abstract: Unavailable

Principal Investigator: Youle, Richard
Grant Number: 5Z01NS002674-20
Title: Engineering Cell Type Specific Toxins

Abstract: Unavailable

Principal Investigator: ZAMORE, PHILLIP D
Grant Number: 5R21NS044952-02
Title: RNAi as a Potential Therapy for ALS

Abstract: Unavailable

Principal Investigator: ZIGMOND, MICHAEL J
Grant Number: 5P50NS019608-20
Title: Neuroprotection and early detection in PD

Abstract: Parkinson's disease (PD) poses a serious threat to the health of a large segment of our society. This is an extensively revised renewal application for a Program Project Grant now in its 18th year. During much of the history of the PPG, we have focused on the compensatory changes that underlie the preclinical phase of PD. That line of investigation will continue, while at the same time we will also add two new foci: first, the development of neuroprotective strategies and, second, the detection of PD it its preclinical phase. Neuroprotection: This will now provide the principal long-term focus of the entire PPG. Our approach derives from recent evidence from our labs indicating that the contralateral motor neglect and loss of DA normally following unilateral damage to the nigrostriatal DA projection can be ameliorated by forced use of the contralateral limb. We hypothesize that forced execution of a motor act that is otherwise compromised by PD is neuroprotective, and that this results from an interaction between the motor act, injury, and concomitant increase in the availability of one or more trophic. We will explore this hypothesis using our 6-hydroxydopamine (6-OHDA) rat model. Our work will involve studies of the role of trophic factors (e.g., GDNF, BDNF, and FGF2), estrogen, and aging, as well as anatomical studies to differentiate between protection, rescue and sprouting (Project 1: M. Zigmond, PI). We also use multineuron recording in awake animals to examine the effect of forced use on the functioning of the basal ganglia more broadly (Project 2, D. Woodward, PI). Compensation: In the past, our studies of compensation have focused our studies on adaptations within the nigrostriatal dopamine (DA) system. Our multineuron recordings will now allow us to explore adjustments within other components of the basal ganglia (Project 2: D. Woodward, PI). Early detection: For neuroprotective strategies to be most effective, it is likely that they must be applied as early in the course of the disease as possible. In this respect, the compensatory changes noted above represent a problem to be overcome through the development of diagnostic tests that can detect PD before the emergence of gross neurological deficits. To do so we will develop a multi-dimensional clinical test battery, using PET imaging as the ultimate criteria for nigrostriatal damage (Project 3, N. Bohnen, PI). We believe that by combining a variety of basic, translational, and clinical approaches we will make significant progress toward the development of a therapeutic approach to PD.-

Principal Investigator: ZIGMOND, MICHAEL J
Grant Number: 3P50NS019608-19A1S1
Title: Neuroprotection and early detection in PD

Abstract: Unavailable